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SURVEY, COLLECTION, ISOLATION AND IDENTIFICATION OF ISOLATES OF *PYRICULARIA ORYZAE* CAUSING RICE BLAST IN SOUTHERN KARNATAKA BY USING HOST DIFFERENTIAL LINES

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ABSTRACT

A survey was conducted during kharif 2015-16 in southern Karnataka, to assess the percent severity and incidence of rice leaf and neck blast. The freshly infected leaves were randomly collected from all the talks viz., Mandya, Maddur, Nagamangala, Shrirangapattana, Pandavapura, Krishnarajpet, and Malavalli of Mandya district of Karnataka, from these samples, the causal organism was isolated by following tissue isolation method and monoconidial isolation. Among all the talk, the highest percent disease severity of the rice leaf blast was recorded (38.92%) in Krishnarajpet Taluk and lowest incidence was recorded (27.39%) in Maddur Taluk. Among all the talk, the highest percent disease incidence of neck blast was recorded (20.61%) in Shrirangapattana Taluk and lowest incidence was recorded (13.04%) in Maddur Taluk. Screening of promising host differential lines against rice blast was carried out to identify the races of P. oryzae. Among twenty five host differential lines of paddy, 2, 3, 4, 10, 12, 13 and 22 host differential shows resistant reaction. Host differential lines 6, 7, 8, 9, 11, 14, 23, 24 and 25 show moderate resistances. Host differential lines 15, 16 and 17 exhibited susceptible reaction.

KEYWORDS: Rice Blast, Tissue Isolation, Monoconidial Isolation, Severity, Incidence & Host Differential Lines

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Original Article

INTRODUCTION

Rice is a cereal grain, belonging to the grass family of Poaceae, and native to the deltas of the great Asian rivers, the Ganges, the Chang (Yangtze) and the Tigris and Euphrates. The rice plant grows from 2 to 6 ft tall, with a round, jointed stem, long pointed leaves and edible seeds borne in dense heads on separate stalks. Rice is one of the most cultivated grain crops in India as well as in Asian countries, and a staple diet of the major part of India. India is an important center for rice cultivation and consumption. South India consumes more rice than any part of the country. India stands in second position after China in the production of rice. The world's estimated rice production is 496.0 million metric tons during 2016 (Anon, 2016). India is the largest rice growing country, accounting for about one third of the world acreage under the crop. In India's annual rice production is 103.6 million tons during 2016 (Anon, 2016).

Rice is a nutritional staple food, which provides instant energy as its most important component is carbohydrate (starch). On the other hand, rice is poor in nitrogenous substances with an average composition of these substances being only 8per cent and fat content or lipids only negligible, i.e. 1 per cent and due to this reason it is considered as a complete food for eating. Rice flour is rich in starch and is used for making various food materials. It is also used in some instances by brewers to make alcoholic malt. Likewise, rice straw mixed with other materials is used to produce porcelain, glass and pottery. Rice is also used in manufacturing of paper pulp

and livestock bedding. Rice has shaped the culture, diets and economic of thousand of millions of people. For more than half of the humanity, "rice is life". Considering its important position, the United Nation designated year 2004 as the "International Year of Rice.

In south Indian region, comprises of Karnataka, Andhra Pradesh, Kerala and Tamil Nadu, Rice is mainly grown in deltaic tracts of Godavari, Krishna and Cauvery rivers and the non-deltaic rain fed area of Tamil Nadu and Andhra Pradesh. Rice is grown under irrigated condition in deltaic tracts.

MATERIAL AND METHODS

Roving survey was conducted during *kharif* 2015-16, around Mandya district of southern Karnataka to assess the percent severity, and incidence of rice leaf and neck blast.

Collection of Infected Specimen and Isolation of the Fungus

The freshly infected leaves were randomly collected from all the seven talks *viz.*, Mandya, Maddur, Nagamangala, Shrirangapattana, Pandavapura, Krishnarajpet, and Malavalli of Mandya district of Karnataka, and were used for isolation by adopting the following methods.

Tissue Isolation Technique

Rice leaves with blast symptoms were first washed in tap water, and then cut into small bits of 2mm size, containing the blast lesion along with a portion of healthy tissue surrounding the lesion. These bits were surface sterilized with 0.1 percent sodium hypochlorite solution for two min, followed by three changes of sterilized distilled water. These bits were transferred into petri dishes containing 15 ml solidified PDA under aseptic conditions, and incubated at 28 ± 1^0C and watched for the growth of colony, regularly. For the growth of the pathogen from the tissue, or for any contamination, up to fourteen days investigation was done. After fourteen days of incubation, a small loop of fungal culture from the colonies was picked, and put on a clean slide containing a drop of lacto phenol. The slide was observed under low and high power objectives for the presence of pyriform conidia.

Single Spore Isolation Technique (Monoconidial Isolation)

Well developed lesions were identified, excised and washed in running tap water. The leaf bits were surface sterilized with 0.1 sodium hypochlorite for 2 min, and then washed three times with sterile water and allow for speculation on sterilized glass slides, by incubating in a moist chamber at 28 ± 1^0C for 48 h. Well sporulated lesions were placed in double distilled water, in the test tubes and vortexed for 1 min. About 1 ml of spore suspension was added to sterilize plates, and 2% agar was added. Single spore was located and picked up microscopically. Each spore was eventually transferred to solidified PDA slants. The slats were incubated at 28 ± 1^0C for 2 days and stored at 4^0C for further use. (Goh, 1999)

Identification of Races of *Pyricularia Oryzae* by using Host differentials

The experiment was conducted during *Rabi* 2015-16 at Krishi Vigyan Kendra Farm, V.C. Farm, Mandya, screening of promising host differential lines, against the blast of rice caused by *P. oryzae* and its races.

Screening work was carried out by pot culture technique. The sterilized soil, sand and FYM were mixed in 1:1:0.5 proportion (w/w basis) and filled in disinfected earthen pot. Seeds were shown on pots; after 15 days, when two leafy

stages were appeared the spore suspension of different isolates was inoculated. The pots were covered with polyethylene bags for 4 days, after that, the polyethylene bags were removed and then, observation was recorded based on disease scale (0-9) (Table 1) and disease grade (Table 2).

A total of 25 host differentials was sown during *Rabi* 2015-16, and were evaluated for the reaction of leaf blast. Each germplasm entry was shown in two rows at a distance of 30 cm long and 10 cm apart, between the two test entries, and two lines of local susceptible variety was sown after every 25 lines.

The screening of germplasm for their reaction to leaf blast was carried under greenhouse conditions. Scoring for leaf blast was done at nursery stage as per the scale given by Nagaraja *et al*., (2007).

Table 1: Scale for Rice Blast Caused by *P. Oryzae*

Scale	Description
0	No infection
1	Pin head spots less than 1 per cent leaf area affected
2	Pin head spots 5-10 per cent leaf area affected
3	Typical blast spots with grey centre 5-25 per cent leaf area affected
4	25-50 per cent leaf areas affected
5	Large spots with grey centre more than 50 per cent leaf area affected

On the basis of disease grade obtained the germplasm were grouped into the following categories.

Table 2: Disease Grade and Varietal Reaction for Rice Blast Caused by *P. Oryzae*

Disease Grade	Varietal Reaction
0	Immune (I)
Up to 1	Highly resistance (HR)
>1.1 to 2.0	Resistant (R)
>2.1 to 3.0	Moderately resistant (MR)
>3.1 to 4.0	Moderately susceptible (MS)
>4.1 to 5.0	Susceptible (S)
>5	Highly Susceptible (HS)

RESULTS

Survey on Severity of Rice Leaf Blast (*P. Oryzae*)

Roving survey was conducted during *kharif* 2015-16 around the Mandya district of Karnataka, to assess the percent severity of blast disease of rice and the results of the data is presented in table 8, figure 1

The symptoms in the field of rice were recognized by broadly spindle shaped spots, with the pale ashy center and brownish red margins seen on the leaf. In case of severe infection, several such spots coalesce and the lamina are destroyed (Plate 1)

Average per cent severity of rice leaf blast i.e. 30.31 per cent was recorded in Mandya Taluk. Highest percent disease severity was recorded (43.27%) in V.C. Farm village and the lowest was recorded (18.68%) in Madla village.

Average percent disease severity of rice leaf blast i.e. 27.39 per cent was recorded in Maddur Taluk. Maximum percent disease severity was recorded (41.88%) in Sompura and minimum was recorded (16.82%) in Volagarahalli village.

Average percent disease severity, i.e. 33.98 per cent was recorded in Nagamangala Taluk. Maximum disease severity (45.61%) was recorded in Shivanhalli village and the minimum was recorded (27.36%) in Hulikere village.

Average percent disease severity, i.e. 38.31 per cent was recorded in Shriranagapattana Taluk. Maximum disease severity was recorded (45.88%) in Mallenhalli village and the minimum was recorded (33.50%) in Kodalakuppe village.

Average percent disease severity, i.e. 33.48 per cent was recorded in Pandavapura Taluk. Maximum disease severity of was recorded (41.23%) in Haranahalli village and the minimum was recorded (26.75%) in Berankoppe village.

Average percent disease severity, i.e. 38.92 per cent was recorded in Krishnarajpet Taluk. Maximum disease severity of was recorded (46.88%) in Laxmipura village and the minimum was recorded (31.23%) in Hosaholalu village.

Average percent disease severity, i.e. 33.85 per cent was recorded in Malavalli Taluk. Maximum disease severity of was recorded (41.60%) in Markalu village and the minimum was recorded (26.82%) in Haladasanahalli village.

Survey on Incidence of Rice Neck Blast (*P. Oryzae*)

Roving survey was conducted during 2015-16 around the Mandya district of Karnataka, to assess the incidence of neck blast disease of rice, and the results of the data is presented in table 3, figure 1.

The symptoms in the field of rice were recognized by broadly spindle shaped spots, with the pale ashy center and brownish red margins are seen on the neck. In case of severe infection, several such spots coalesce and the lamina are destroyed (Plate 1)

The average per cent incidence of rice neck blasts i.e. 15.83 per cent in Mandya talk was. Highest per cent incidence was recorded (24.25%) in V.C. Farm village and lowest recorded (9.68%) in Madla village.

Average percent disease incidence, i.e. 13.04 per cent was recorded in Maddur Taluk. Maximum disease incidence was recorded (23.23%) in Sompura and minimum disease incidence was recorded (7.62%) in Volagarahalli village.

Average percent disease incidence, i.e. 16.73 per cent was recorded in Nagamangala Taluk. Maximum disease incidence was recorded (22.32%) in Shivanhalli village and minimum disease incidence was recorded (11.28%) in Hulikere village.

Average percent disease incidence, i.e. 20.61 per cent was recorded in Shriranagapattana Taluk. Maximum disease incidence was recorded (28.96%) in Mallenhalli village and minimum disease incidence was recorded (13.72%) in Kodalakuppe village.

Average percent disease incidence, i.e. 16.41 per cent was recorded in Pandavapura Taluk. Maximum disease incidence was recorded (21.63%) in Haranahalli village and minimum disease incidence was recorded (10.82%) in Berankoppe village.

Average percent disease incidence, i.e. 20.38 per cent was recorded in Krishnarajpet talk Maximum disease incidence was recorded (29.85%) in Laxmipura village and minimum disease incidence was recorded (14.28%) in Hosaholalu village.

Average percent disease incidence, i.e. 33.85 per cent was recorded in Malavalli Taluk Maximum disease incidence was recorded (41.60%) in Markalu village and minimum disease incidence was recorded (26.82%) in Haladasanahalli village.

Among all the talk surveyed (Figure 1) (Table 3) highest average percent disease severity of the rice leaf blast was

recorded (38.92%) in Krishnarajpet Taluk and lowest incidence was recorded (27.39%) in Maddur Taluk. The village with highest percent disease severity was recorded (46.88%) in Laxmipura village of Krishnarajpet Taluk and lowest per cent disease severity was recorded (16.82%) in the Volagarahalli village of Maddur Taluk.

Among all the talk surveys (Figure 2) (Table 4) highest average percent disease incidence of neck blast was recorded (20.61%) in Shrirangapattana Taluk and lowest incidence was recorded (13.04%) in Maddur Taluk. The village with highest percent disease incidence was recorded (28.96%) in the Malenhalli village of Shrirangapattana Taluk and lowest per cent disease incidence was recorded (7.62%) in the Volagarahalli village of Maddur Taluk.

Table 3: Percent Severity and Incidence of Rice Leaf and Neck Blast Disease around Mandya District

Name of Taluk	Name of Village	Varieties	Leaf Blast (%)	Mean (%)	Neck Blast (%)	Mean (%)
1. Mandya	Dudda	MTU 1001	34.71	30.31	18.71	15.83
	Shivalli	MTU 1001	35.53		19.56	
	Hullenahalli	MTU 1001	38.32		17.94	
	Madla	BR 2655	18.68		9.68	
	Holalu	BR 2655	21.40		12.45	
	Mallanayakanakatte	Thanu	26.83		14.85	
	Gandalu	BR 2655	23.74		9.21	
	V.C.Farm	Jyoti	43.27		24.25	
2. Maddur	Desahalli	Jaya	24.93	27.39	11.12	13.04
	Sivapura	Jaya	38.71		14.65	
	N.I.Doddi	Thanu	28.37		8.68	
	Sompura	MTU 1001	41.88		23.23	
	Volagarahalli	BR 2655	16.82		7.62	
	Bellur	Jaya	37.74		18.52	
	Somanhalli	BR 2655	19.80		11.47	
	Haralahalli	BR 2655	17.51		9.58	
	Shankarapura	Thanu	28.63		12.92	
	Hosahalli	BR 2655	19.55		12.68	
3. Nagamangala	Shivanhalli	Jyoti	45.61	33.98	22.32	16.73
	Dondemadanahalli	Jyoti	34.95		17.88	
	Vaddarahalli	Jaya	32.52		19.52	
	Hulikere	Thanu	27.36		11.28	
	Javaranahalli	Thanu	29.47		12.65	
4. Shrirangapattana	Rampura	MTU 1001	43.52	38.31	23.56	20.61
	Doddegowdan koppalu	Jaya	39.27		19.52	
	Mallenhalli	MTU 1001	45.88		28.96	
	Huknalli	Thanu	36.34		20.04	
	K.shettihalli	BR 2655	28.93		13.72	
	Kudalakuppe	MTU 1001	33.50		16.39	
	Naguvahalli	MTU 1001	40.73		22.12	
5. Pandavapura	Chikkabyadarahalli	Mangala	39.53	33.48	17.32	16.41
	Haranahalli	MTU 1001	41.23		21.63	
	Pattasomanhalli	Thanu	28.50		14.69	
	Harohalli	Jaya	34.30		16.54	
	Rajmundanlli	Jaya	32.32		15.85	
	Gumnalli	Jyoti	35.29		13.24	
	Berankoppe	Thanu	26.75		10.82	
	Vasuru	Jyoti	33.53		19.54	
	Devegowdankapalu	Jyoti	31.50		15.23	
	Narayanpura	Jyoti	37.92		19.28	
6. Krishnarajpet	Bommenahalli	Rajmudi	44.52		24.22	20.38
	Harinahalli	Thanu	39.27		18.23	

Name of Taluk	Name of Village	Varieties	Leaf Blast (%)	Mean (%)	Neck Blast (%)	Mean (%)
	Laxmipura	MTU1001	46.88		29.85	
	Ichanahalli	Rajmudi	36.34	39.92	17.56	
	Akkihebbala	Jaya	33.50		14.28	
	Hosaholalu	Jaya	31.23		16.98	
	Machahalli	MTU 1001	40.73		21.58	
7. Malavalli	Ravani	Minilong	36.5		14.32	14.73
	Haladasanahalli	BR 2655	26.82		9.56	
	Ragibommanahalli	Thanu	32.68		15.76	
	Markalu	Minilong	41.60		18.65	
	Kanhalli	Jaya	35.52		14.28	
	Manjunuru	Jaya	37.32		19.65	
	Halasahalli	Jyoti	30.11	33.85	13.38	
	Jarajanpura	Jaya	33.35		14.34	
	Akkomorkopalla	Jaya	33.42		12.68	

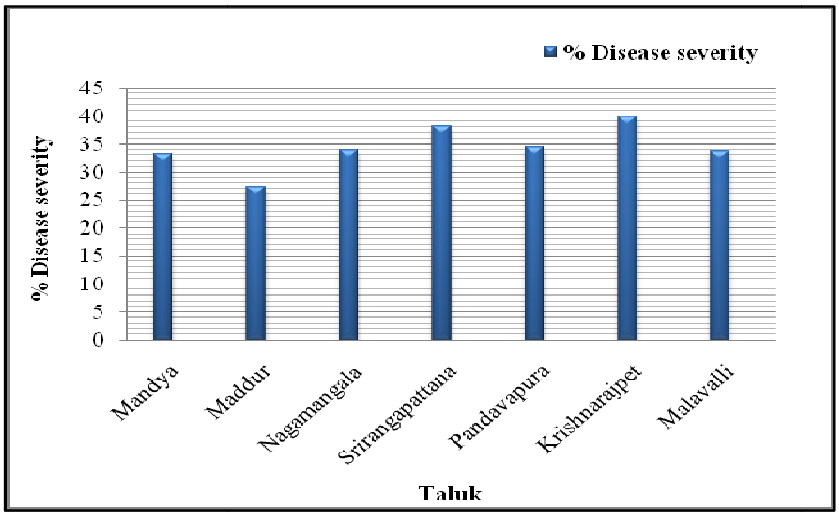

Figure 1: Per Cent Disease Severity of Rice Leaf Blast in different Taluks of Mandya District

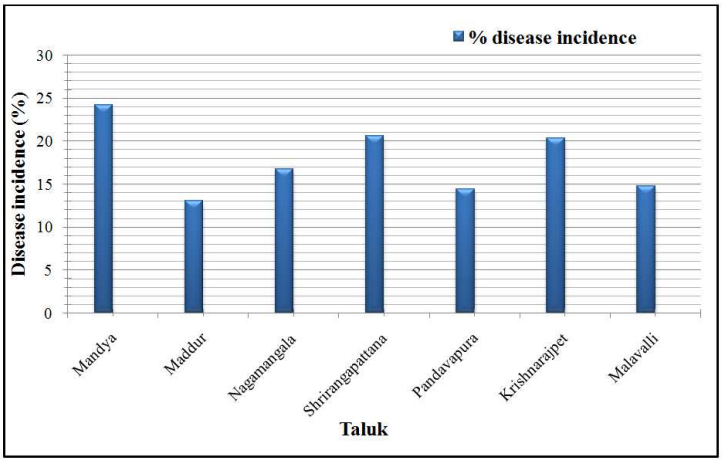

Figure 2: Per Cent Disease Incidence of Rice Neck Blast in different Taluks of Mandya District

Plate 1: Typical Symptoms of All Isolates of the Rice Leaf Blast

Collection, Isolation and Identification of *P. Oryzae*

The infected leaf samples having typical symptoms of blast on rice caused by *P. arise* were collected from all the seven talks of Mandya district (Plate 1). From these samples, the causal organism was isolated by following tissue isolation method. Further, monoconidial isolation was done to obtain pure cultures of all isolates, as detailed in 'material and methods. The fungus was sub-cultured on PDA slants, and stored in refrigerator at 4^0C. For further studies, the original culture of each isolates was revived once in 30 days.

Identification of Races of *P. Oryzae* by using Host differential

The present study was made to locate the resistant sources to *P. Rise* in rice, as the utilization of resistant varieties is a classical approach to prevent losses caused due to the disease. This approach is novel in the management of disease, as it involves no or less cost of production.

Among twenty five host differential lines of paddy, 2, 3, 4, 10, 12, 13 and 22, host differential shows resistant reaction. Host differential lines 6, 7, 8, 9, 11, 14, 23, 24 and 25 show moderate resistance. Host differential lines 15, 16 and 17 exhibited susceptible reactions (Table 6).

Table 4: Isolates of *P. Oryzae*

Sl. No.	Location	Plant Part	Isolates Designation
1	Mandya	Leaf	I_1
2	Maddur	Leaf	I_2
3	Nagamangala	Leaf	I_3
4	Shrirangapattana	Leaf	I_4
5	Pandavapura	Leaf	I_5
6	Krishnarajpet	Leaf	I_6
7	Malavalli	Leaf	I_7

Table 5: Identification of Races of *P. Oryzae* by using Host differential Lines of Rice during *Rabi* 2015-16

Host Differential Lines	Reaction							No. of Isolates
	Isolates							
	I-1	I-2	I-3	I-4	I-5	I-6	I-7	
1	S	R	S	S	R	R	R	4
2	S	R	S	S	R	R	R	4
3	S	R	R	S	R	S	R	6
4	R	R	R	R	R	S	R	6
5	S	R	R	R	R	R	R	6
6	R	S	R	R	S	R	R	5
7	R	S	R	R	S	R	R	5
8	S	R	R	R	R	R	S	5
9	S	R	R	R	R	S	R	5
10	R	R	R	R	S	R	R	6
11	R	S	R	R	S	R	R	5
12	R	S	R	R	R	R	R	6
13	R	R	S	R	R	R	R	6
14	R	R	S	R	S	R	R	5
15	S	R	S	R	S	S	R	3
16	S	S	R	R	R	S	S	3
17	R	S	R	S	R	S	S	3
18	R	R	R	S	R	S	S	4
19	S	R	S	S	R	R	R	4
20	S	S	S	R	R	R	R	4

Table 5: Contd.,								
21	R	S	S	R	R	S	R	4
22	R	R	R	R	R	S	R	6
23	R	R	R	S	S	R	R	5
24	S	R	R	S	S	R	R	5
25	S	R	R	S	R	R	R	5

Grades Reaction

0 = Immune (I),

1-2 = Highly Resistant (HR),

3-4 = Resistant (R),

5-6 = Susceptible (S)

7-9 = Highly susceptible (HS)

DISCUSSIONS

Survey, Isolation and Proving Pathogenicity of *P. Oryzae*

A survey of the disease prevalence of rice blast was undertaken in seven talks of Mandya district, Karnataka *viz.*, Mandya, Maddur, Nagamangala, Shrirangapattana, Pandavapura, Krishnarajpet and Malavalli Taluk. Infected sample showing typical blast symptoms on leaf, neck and nodal portion was collected. The culture of each location was considered as an individual isolates.

During survey, highest average per cent disease severity (38.92%) was recorded in Krishnarajpet Taluk (I_6), whereas, lowest percent severity (27.39%) was recorded in the Maddur Taluk (I_2). Morgan Hossain (2000) and Anwar *et al.*, (2009) also surveyed for the percent severity of rice blast. Morgan Hossain (2000) recorded 61.66 percent disease incidence in Uttar Kannada due to blast caused by *P. oryzae*. However, the present study shows that the maximum disease severity (38.92%) was recorded in Krishnarajpet Taluk (I_6). Hence, the variation in disease incidence among different talks is attributed to the varied climatic conditions and edaphic factors like soil temperature, soil moisture and soil pH.

The sample of rice leaves affected by blast disease was collected from all the talk of Mandya district of Karnataka. The pathogen isolated from diseased leaf showing typical symptoms was identified, as *P. oryzae* on the basis of morphological characters. Pure culture was maintained in PDA medium through hyphal tip isolation technique. The isolation of rice blast fungus observed were in agreement with the description, Dhua (1986), Xia *et al.,* (1993), Goh (1999) and Lima and Duclos (2001).

Pathogenicity test was carried out under pot culture conditions in glasses by inoculating with pathogenic culture of *P. oryzae*. Infected plant leaf showed gray at the center, has a dark border and it is spindle-shaped lesions. The symptoms of rice blast observed were in agreement with the description of Ghose *et al.*, (1960); Pinnschmidt *et al*., (1994); Padmanabhan (1974) and Manibhushan Rao (1994). Hence, pathogencity tests showed typical symptoms, when inoculated with pathogenic culture of *P. oryzae*. Re-isolation of the fungus from such affected leaf tissue in variably yielded pathogenic culture of *P. oryzae.* Hence, the present study clearly indicated that *P. arise* is involved in causing blast disease in rice.

Identification of Races of *P. Oryzae* by using Host differential

The present study was made to locate the resistant sources to *P. Rise* in rice, as the utilization of resistant varieties is a classical approach to prevent losses caused due to the disease. This approach is novel in the management of disease, as it involves no or less cost of production.

Among twenty five host differential lines of paddy, 2, 3, 4.10, 12, 13 and 22, the host differentials show resistant reaction. The host differential lines 6, 7, 8, 9, 11, 14, 23, 24 and 25 show moderate resistances. Host differential lines 15, 16 and 17 exhibited susceptible reaction.

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